Description

RECOGNITION LAYERS MADE OF HYDROGEL BASED ON POLYACRYLAMIDE FOR USE IN BIOSENSOR TECHNOLOGY

The present invention relates to an Immobilization layer for biosensors as well as to its use to create biosensor recognition layers, especially for creating what are known as DNA chips.

Biosensors, in which a biological recognition system is linked to a physical transducer, are increasingly being used in modern biological analysis technology but also in medical diagnostics. Recognition systems are taken to mean topological recognition molecules such as antibodies, enzymes, nucleo acids and such like which are bound via what is known as an immobilization layer to a carrier (transducer). Calorimetric, piezoelectric, optical and electrochemical principles are primarily used as transducers.

The recognition systems or originally the immobilization layers respectively, are immobilized in this case mostly in approximately two-dimensional layers on the transducer systems. The recognition molecules can be immobilized by covalent bindings, by an affinity reciprocal effect but also by hydrophile/hydrophobe reciprocal effects. For reasons of stability covalent bindings are preferred, however the formation of stable complexes, for example Biotin/Avidin are also successfully used. A good overview of the structure of approximately two-dimensional biological recognition layers can be found in I. Willner, E. Katz:"Redox protein layers on conductive carriers - system for bioelectronic applications" in Angew. Chem. (Applied Chemistry) 2000, 112, pp. 1230-69.
With transducer surfaces which contain NH or OH groups the biological function carriers, i.e. the recognition molecules, are frequently immobilized by Alkoxysilanes which contain so-called

linker groups, that is with the aid of Cyanurchloride or Carbodiimide. To equip transducer surfaces containing gold, recognition molecules labeled with thiolalkyl are used which are immobilized via sulfur-gold bonds in the form of so-called self-assembly-layers on the transducer surface. An interesting approach to the immobilization of Nuclein acids on transducer surfaces is the photochemically supported synthesis of Affymetrix (light-directed spatially addressable parallel chemical synthesis, S.P.A. Fodor et al.,Science 251, 767-773 (1991)).

To increase the sensitivity of biosensors as well as to optimize the reproducibility of the measurement results obtained by doing so, it makes sense to use three-dimensional immobilization layers for the biological recognition molecules. The German company Schleicher & Schüll GmbH is offering DNA chips under the name FASTTM Slides, in which the capture oligos are immobilized in a three-dimensional nitrocellulose membrane (BioMolecular Screening, Catalog 2001, intern. Edit. Schleicher & Schüll).

In WO 00/43539 the construction of a three-dimensional DNA recognition layer through immobilization of the DNA capture probes in the form of polymer brushes is described.

Timofeev et al. describe a chemically modified radically cross-linked polyacrylamide which can be used for example for the immobilization of capture oligos (E. N. Timofeev et al.,Regioselective Immobilization of Short Oligonucleotides to Acrylic Copolymer Gels, Nucleic Acids Research, 1966, Vol.24, No. 16, 3142-3148) . Here Amino or Aldehyde groups are used coupling groups in the hydrogel. Aldehyde or Amino functionalized capture oligos can be immobilized covalently at these coupling groups under reductive reaction conditions. This means however that, as well as

the actual coupling reaction between Amino and Aldehyde group or vice versa, an additional reduction layer is required using reduction means. Further methods described by Timofeev et al. for chemical activation of the cross-linked Polyacrylamide also require additional reaction steps in the Polymer matrix.

The object of the present invention is the creation of a hydrophilic immobilization layer for biosensor applications based on a hydrogels as well as the use of such immobilization layers for creating recognition layers through covalent coupling in of biological recognition molecules.

The present invention achieves this object by using radically cross-linked or photostructured hydrogels as the immobilization layer. Such hydrogels are described in the German Patent Application "Radically cross-linkable composition for creating a hydrogel layer" or "Photostructurable composition for creating a hydrogel layer" (File reference yet known) written by the applicant.

The object of the present invention is thus simply a hydrophilic immobilization layer for biosensors made of a radically cross-linked hydrogel based on Polyacrylamide, where the initial composition comprises Acrylamide, cross-linkers, radical linitiators, at least one comonomer with reactive linker groups and where necessary softeners or other additives.

The object of the present compound is also a hydrophilic immobilization layer made of a photostructured hydrogel based on Polyacrylamide, where the initial compound comprises Acrylamide, cross-linking means, photo initiators, at least one film binder, at

least one comonomer with reactive linker groups and where necessary softeners or other additives.

The inventive systems allow the construction of sensor arrays with biological recognition molecules in a three-dimensional matrix at a high level of integration density.

Preferred embodiments or compositions of the hydrophilic immobilization layers are produced by the subclaims 3 to 10.

Further components which guarantee the mixability of the monomers and the initiators can be added to the compositions if necessary. Commercially-available additives can be used to reduce the surface tension.

After layering on a transducer system and thermal or photo cross-linking or photopolymerization or photostructuring or polymerization structuring a water-swellable hydrogel is obtained in which, by using the linker groups, topological or chemical recognition molecules for analytical or diagnostic applications can be coupled in while retaining their functional capabilities. The object of the present invention is consequently also to use the immobilization layers to produce biosensor recognition layers through (covalent) coupling in or Immobilizing off chemical or biological recognition molecules, where the recognition molecules are preferably capture oligonucleotides.

Basically the initial composition for creating the hydrogel layer (immobilization layer) can be applied with all modern layering technologies to the suitable carriers. Preferably however spin coating as well as dispensing is employed.

The properties of the hydrogel layer to be created as regards hydrophile, cross-linking density, water-swellability, etc. can be varied over wide ranges by the type of initial components used, their relationship to each other and in the final analysis the type of layer formation.

The hydrogel matrix can be adapted to the biological recognition molecules to be used, especially with regard to the cross-linking density. The cross-linking density is controlled by the type and concentration of the cross-linking molecules used, such as Acryl and/or Methacryl compounds, especially Methylenbis(meth)acrylamide and/or Dimethacrylic acid esters, such as Tetraethyleneglycoldimethacrylate.

The hydrogel mixture can also be adapted to the coating process preferred for the specific application purpose.

For spin-coating one of the methods in question is to use a polymer film former such as Polyvinylpyrrolidon, Polyacrylamide and/or Polyhydroxymethacrylate. Another is to use high-boiling point solvents such as Ethylene glycol, for the hydrogel mixture, which do not vaporize completely on spin coating and thus remain as softeners in the layer. The residual solvent content can then be explicitly further reduced by a prebake step before cross-linking and thereby the polymerization yields or the resulting layer thickness controlled. If necessary further softener systems, such as Diethyleneglycol and/or Triethyleneglycol, can be added.

For layer formation by dispensing the hydrogel mixture is applied in solution depending on the transducer dimensions in drops of a few microliters up to one nanoliter in size. For dispensing high-temperature solvents which exhibit a sufficiently long lifetime of

the drop at the tip of the dispensing channels are used. This means that the dosing and deposition of the drop are reproducable. On the other hand the boiling point of the solvent may not be too high in order to allow a sufficiently rapid evaporation of the solvent from the deposited drop.
If necessary annealing step for controlling the residual solvent content may be required. In accordance with the invention Dimethylformamide and/or Ethyleneglycol are preferably used for dispensing the hydrogel mixture.

The hydrogel mixture can be applied in layer or spot form on transducer or carrier surfaces made of metal, glass, silicon, silicon dioxide, silicon nitride or plastic. Surfaces with a topography that consists of different materials, for example. Interdigital electrode arrays on Silicon nitride as passivization can also be coated. The coating of surfaces thus also includes the coating of inner surfaces of micrchannels or nanotubes. The surfaces to be coated are if necessary coated with an adhesion promoter.

The polymerization and cross-linking of the hydrogel layer is undertaken by thermal or UV initiation. With UV initiation the hydrogel layer can also be structured by contact or proximity illumination through a mask. The hydrogel layer operates here like a negative resist. Ploymerization and cross-linking are undertaken in the radiated area. There is no reaction in the darkened areas. The hydrogel mixture located here is removed from the substrate again in a development step. Auxiliary components such as Polymer film formers or softeners can be removed by extraction from the cross-linked hydrogel layer. This step can under some circumstances be undertaken simultaneously with the actual equipping step.

The biological or chemical recognition systems are preferably applied from an aqueous solution, an aqueous buffer solution or

mixtures of polar solvents with water onto the immobilization layer. They are applied by drops or by spotting/dispensing. In nanotubes or microchannels the solution can also be applied with the biological or chemical recognition molecules to the cross-linked hydrogel layer by transport through the fluid system itself. For precisely targeted loading of measuring spots cross-linked hydrogel spots which are surrounded by a protective ring are advantageously used.

For covalent coupling of the biological or chemical recognition molecules which are provided with a coupling group suitable for the linker group present in the cross-linked hydrogel an annealing step may be required, depending on reactivity. To prevent the hydrogel layer drying out during the coupling reaction operations can be carried out in a climate-controlled chamber. Aminoalkyl groups are especially suitable for coupling to the Epoxide and Maleic acid anhydride linker groups.